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(54) Process for heat treatment of blood coagulation factor VIII.

(57) Heat treatment of an aqueous solution or a fraction containing blood coagulation factor VIII to inactivate hepatitis viruses is carried out in the presence of a stabilizer selected from sugar-alcohols or disaccharides in a concentration of at least 1.5 g per ml of the aqueous solution or fraction.

EP 0 117 064 A2

PROCESS FOR HEAT TREATMENT OF  
BLOOD COAGULATION FACTOR VIII

1           This invention relates to a process of heat  
treatment to inactivate the viruses suspected of contaminat-  
ing an aqueous solution or fraction containing human  
blood coagulation factor VIII.

5           The blood coagulation factor VIII (hereinafter  
referred to briefly as factor VIII), also called anti-  
hemophilia factor A, is one of the blood coagulation  
factors contained in the plasma. A diathesis due to  
congenital deficiency of the factor VIII is a disease called  
10 hemophilia A. In a patient suffering from this disease,  
the blood coagulation reaction necessary in the event  
of hemorrhage will not become complete and even a slight  
wound leads to a large amount of bleeding.

          The factor VIII preparations are widely in use  
15 for the purpose of treatment and prevention of hemorrhage  
by supplying the factor VIII to the patients suffering  
from congenital deficiency or diminution of factor VIII.  
In recent years, however, the onset of serum hepatitis  
accompanied with the transfusion of blood or blood  
20 components has become one of the serious social problems.  
It has been made clear that the cause for the serum  
hepatitis is a hepatitis virus. The human serum protein  
preparations prepared by the fractionation of blood  
plasma also involves the problem of hepatitis incidence.  
25 The factor VIII, which is the subject of this invention,

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1 is also one of the human serum protein preparations and  
is suspected of the contamination with hepatitis viruses.

It was found in an effort to solve the problem  
of hepatitis-viral infection that the infective activity  
5 of hepatitis viruses in serum preparations, in general,  
particularly in albumin preparations, may be controlled  
by the heat treatment at 60°C for 10 hours without causing  
denaturation of the albumin. Since the albumin prepara-  
tions which undergone such a heat treatment has been  
10 clinically used as a drug with safety, the heat treatment  
at 60°C for 10 hours is now being adapted to other human  
serum protein preparations. In order to apply such a heat  
treatment, the substance being treated must, of course,  
be stable to the treatment. The factor VIII, which is  
15 the subject of this invention, however, loses its activity  
to a marked degree when it is heated in an aqueous solution  
at 60°C for 10 hours.

Regarding the heat treatment of factor VIII,  
H.J. Weiss et al. reported in 1965 that a citrated plasma  
20 adjusted to pH 6.9 retained 90% of its activity of factor  
VIII after the plasma had been heated at 37°C for 18 hours  
(Thromb. Diath. Haem., Vol. 14, p. 32). More recently,  
two reports were published one after another on a process  
of heat-treating factor VIII in a solution in the presence  
25 of a sugar. The one is "blood coagulation factors and  
a process for the production thereof" [Japanese Patent  
Application "Kokai" (Laid-open) No. 145,615/80] and the  
other an European patent application entitled "Pasteurized

1 therapeutically active protein compositions" (EP 35,204 A2).

The necessary condition for heat treatment is the presence of saccharose alone or in combination with an amino acid in a concentration of 20 to 60% (W/W) in terms of

5 saccharose.

The present invention is predicated upon the finding that in heat-treating factor VIII to inactivate hepatitis viruses, the heat stability of said factor is improved to a marked degree by using as a stabilizer a  
10 sugar-alcohol or a disaccharide in a high concentration.

An object of the present invention is to provide a novel process of heat treatment, wherein the thermal stability of the human blood coagulation factor VIII is improved, in inactivating hepatitis viruses contaminated  
15 in an aqueous solution or fraction containing said factor VIII.

Other objects and advantages of the present invention will become apparent from the description which follows.

20 According to the present invention, there is provided a process for the heat treatment of factor VIII to inactivate hepatitis viruses suspected of contamination, which comprises heating a solution or fraction containing said factor VIII preferably at 30° to 80°C for 3 to 24  
25 hours or at 90°C for 1 minute in the presence of a stabilizer selected from sugar-alcohols or disaccharides in a high concentration of 1.5 g or more per ml of said aqueous solution or fraction containing the factor VIII.

1           As examples of stabilizer used in this inven-  
tion, mention may be made of sorbitol and mannitol among  
sugar-alcohols; saccharose, maltose and lactose among  
disaccharides. The minimum amount to be used of the  
5 stabilizer is 1.5 g per ml of the aqueous solution or  
fraction containing factor VIII, which corresponds to  
60% (W/W) in ultimate concentration, assuming the specific  
gravity of the solution to be 1. When sorbitol or  
saccharose is used as the stabilizer, its total amount  
10 may be reduced by the joint use with a neutral amino acid  
such as glycine.

The factor VIII to be treated according to this  
invention is subject to no restriction so long as it is  
of the human origin. Factor VIII is contained chiefly  
15 in the human plasma and the methods for its separation  
and purification using the human plasma as the starting  
material are already known (U.S. Patent 3,631,018, PEG  
fractionation method; U.S. Patent 3,652,530, Glycine  
fractionation method; Japanese Patent Publication  
20 No. 1290/1980, Anion-exchange treatment method; Johnson  
A.J. et al., British Journal of Haematology, 21, 21 (1970),  
Co-use of aluminum hydroxide adsorption method and PEG  
fractionation method; Wagner R.B. et al., Thrombosis  
Diathesis Haemorrhagica, 11, 64 (1964), Co-use of aluminum  
25 hydroxide adsorption method and PEG fractionation method).

The solution being heat-treated has a pH of  
generally 5.0 to 10.0, preferably 6.0 to 8.0 and the  
activity of factor VIII contained in said solution is

1 preferably 1 to 50 units/ml.

A series of sample solutions were prepared by adding varied amounts of sorbitol in the range of 0.4 to 2.5 g to 1 ml of a solution containing factor VIII.

5 Each of the resulting sample solutions was heat-treated at 60°C for 2 hours to examine the retention of the activity of factor VIII. The results obtained were as shown in Table 1. Entirely no loss of the factor VIII was observed after the said heat treatment when the sugar  
10 alcohol content is 1.5 g [corresponding to an ultimate concentration of 60% (W/W), assuming the specific gravity of the solution to be 1] or more. The assay of the activity of factor VIII was performed by the method of thrombo-  
plastin formation test [Pool and Robinson, British  
15 Journal of Haematology, 5, 17 (1959)].

Table 1

	Amount of added sorbitol, g					
	0.48	0.79	1.0	1.5	2.2	2.5
Retention of activity, %	10	26	58	100	100	100

In the next experiment, sugar alcohol, disac-  
charide, a mixture of sugar alcohol and neutral amino  
acid, or a mixture of disaccharide and neutral amino  
acid was added to 1 ml of a solution containing factor  
20 VIII. The resulting solution was subjected to heat treat-  
ment at 60°C for 10 hours to examine the retention of

- 1 the activity of factor VIII. The results were as shown in Table 2.

Table 2

Stabilizer and amount added		Retention of activity, %
Sorbitol	1.5 g	58.0
Saccharose	1.5 g	57.0
Sorbitol	1.0 g	47.5
Glycine	0.15 g	
Saccharose	1.0 g	46.5
Glycine	0.15 g	
Sorbitol	1.2 g	43.5
Saccharose	1.2 g	42.5
None		0

The corelation between the degree of purification and the thermal stability of factor VIII is insignificant.

- 5 The stabilizing effect of sugar alcohol or disaccharide remains unchanged when factor VIII of whatever degree of purification is used. As a consequence, the heat treatment for the inactivation of hepatitis viruses may be carried out at any stage of purification of factor VIII.

- 10 The process of this invention is useful as a commercial process for the production of a factor VIII preparation, because according to this invention it is possible to inactivate the hepatitis viruses suspected of contaminating a blood preparation without causing an excessive loss in the activity of factor VIII which is a valuable
- 15

1 principle of blood preparations.

The invention is illustrated below with reference to Examples, but the invention is not limited thereto.

5 Example 1

A solution containing partially purified factor VIII is adjusted to pH 7.0 with 0.2 N hydrochloric acid. Into 1 ml of the solution, was added 2.0 g of sorbitol which was allowed to dissolve by heating at 37°C. The  
10 resulting solution was heated in a water bath at 60°C for 10 hours to inactivate hepatitis viruses. Substantially no precipitate was formed and, hence, the step of precipitate removal was unnecessary. The sorbitol was removed by ultrafiltration and the factor VIII was  
15 concentrated. The retention of the activity of factor VIII was found to be 58%.

Example 2

Into 1 ml of a solution (pH 7.0) of partially purified factor VIII, was added 2.0 g of saccharose and  
20 allowed to dissolve by heating at 37°C. The resulting solution was heated at 90°C for 1 minute in a water bath to inactivate hepatitis viruses. The saccharose was then removed by ultrafiltration and the factor VIII was concentrated. The retention of the activity of factor VIII  
25 was 56%.

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1 Example 3

A cryoprecipitate extract originated from human plasma was adjusted to pH 7.0 with 0.2 N hydrochloric acid. Into 1 ml of the adjusted extract, was added 2.0 g  
5 of sorbitol and allowed to dissolve by heating in a bath at 37°C. The resulting solution was heated in a bath at 80°C for 3 hours to inactivate hepatitis viruses. The sorbitol was removed by ultrafiltration and the factor VIII was concentrated. The retention of the activity of factor  
10 VIII was 58%.

Example 4

Into 1 ml of a cryoprecipitate extract, which had been adjusted to pH 7.0 with 2 N hydrochloric acid, was added 2.0 g of saccharose and allowed to dissolve by  
15 heating in a bath at 37°C. The resulting solution was heated in a bath at 80°C for 3 hours to inactivate hepatitis viruses. The saccharose was then removed by ultrafiltration and the factor VIII was concentrated. The retention of the activity of factor VIII was 57%.

20 In the foregoing Examples 2 to 4, there was no formation of precipitate and, accordingly, the operation of precipitate removal was not needed.

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CLAIMS:-

1. In a process for the heat treatment of a solution or fraction containing blood coagulation factor VIII to inactivate hepatitis viruses, the improvement which comprises carrying out the heat treatment in the presence  
5 of a stabilizer selected from sugar alcohols or disaccharides in a concentration of 1.5 g or more per ml of said solution or fraction.
2. A process according to Claim 1, wherein the stabilizer is sorbitol or saccharose.
- 10 3. A process according to Claim 2, wherein the stabilizer is a combination of sorbitol or saccharose with a neutral amino acid.
4. A process according to Claim 3, wherein the neutral amino acid is glycine.
- 15 5. A process according to Claim 1, wherein the heat treatment is carried out at 30° to 80°C for 3 to 24 hours or at 90°C for 1 minute.